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ALLELE-SPECIFIC GENE EXPRESSION ANALYSIS IN ALFALFA HYBRIDS AND NEOPOLYPLOIDS

ROSELLINI D.*, BOCCHINI M.*, MARCONI G.*, ALBERTINI E.*, VERONESI F.*

*) Department of Agricultural Food and Environmental Sciences, University of Perugia, Italy

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Polyploidization is a major evolutionary force in plants. Our objective is to investigate how gene expression is affected by sexual polyploidization in alfalfa (Medicago sativa L., 2n=4x=32), an important autotetraploid forage crop. By crossing diploid (2n=2x=16) *M. sativa* meiotic mutants we obtained diploid (2x) and tetraploid (4x) full-sib progenies that allow to dissect the effect of polyploidization from that of hybridization on gene expression. In this work, we investigated allele-specific gene expression (ASE) in three 2x and three 4x progenies and their 2x parental plants by RNA-seq, taking advantage of the ASE pipeline of Sequentia Biotech. We also low-coverage sequenced parental genomic DNAs to distinguish maternal and paternal alleles in the progeny.

First, based on a recently published chromosome-level genome assembly of 4x alfalfa, a virtual, linear reference genome of 8 chromosomes was constructed, by concatenating one allele for each protein coding gene separated by 50 Ns. Then, DNA-seq and RNA-seq reads were mapped onto this reference, and SNPs in parental individuals were called using either DNA-seq or RNA-seq data. Only the SNPs located within exons were considered. A total of 480,991 and 239,474 SNPs were identified using DNA-seq and RNA-seq data, respectively.

SNPs between parental genomes in the homozygous state (necessarily heterozygous in the progenies) were selected in order to identify parent-of origin bias in progeny gene expression, by looking at the percentage ratio of the read counts of the reference allele (the allele found in the virtual reference genome) over total (reference + variant) read counts. In case of unbiased expression, 50% of reference and variant alleles are expected in

the progenies. The results showed that parental bias was widespread, but not consistently different between 2x and 4x progenies. The number of variants changing in expression ratio was approximately the same in the comparisons of parents vs 2x or parent vs 4x progenies (6398 and 6538 variants respectively), whereas the difference between 2x and 4x progenies was much smaller (4054 variants).

Finally, to estimate RNA editing, the variants found in DNA-seq analysis between parents were compared with the variants found in RNA-seq analysis of the same individuals. We found a total of 91,867 variants in mRNAs not present in the DNA. A majority of these (55,142) were identified in the male parent, suggesting parent-specific RNA editing.

Our results suggest that, in the limited sample of neopolyploids examined here, consistent parent-of-origin bias in gene expression is not a significant outcome of alfalfa sexual autotetraploidization. However, parentally biased RNA editing might be a significant source of variation and deserves further study.